U.S. HIGH PRODUCTION VOLUME (HPV)

CHEMICAL CHALLENGE PROGRAM

ROBUST SUMMARY

1H-Isoindole-1,3(2H)-dione, 5,5'-[(1-methylethylidene) bis(4,1-phenyleneoxy)]bis[2-methyl-

(Bisphenol A Bisimide; CAS RN 54395-52-7)

Prepared By:

General Electric Company

One Plastics Avenue

Pittsfield, MA 01201

Prepared for:

U.S. Environmental Protection Agency

Washington, D.C., USA

December 22, 2005

Table of Contents

CHEMICAL IDENTITY AND USE INFORMATION

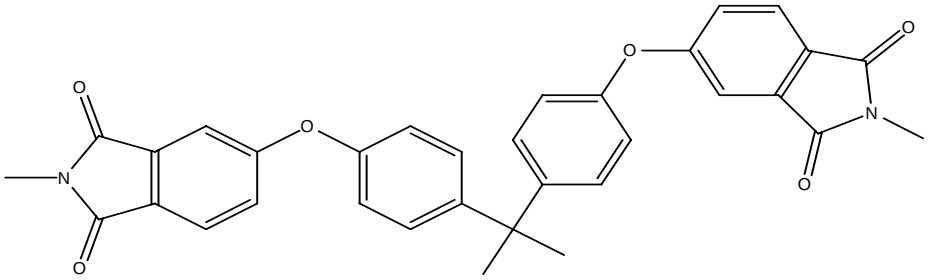
CAS RN

54395-52-7

CHEMICAL NAME

1H-Isoindole-1,3(2H)-dione, 5,5'-[(1-methylethylidene)bis(4,1-phenyleneoxy)]bis[2-methyl-
(hereafter called Bisphenol A Bisimide)

STRUCTURE, MOLECULAR FORMULA, MOLECULAR WEIGHT



Molecular Formula: $C_{33}H_{26}N_20_6$
Molecular Wt.: 546.57

OTHER CHEMICAL IDENTITY INFORMATION

4,4'-((Isopropylidene)bis(p-phenyleneoxy))bis(N-methylphthalimide-)
Bisphenol A Bisimide
Bisphenol A Diimide
N,N'-Dimethyl-2,2-bis(4-(3,4-dicarboxyphenoxy)phenyl)propane diimide
BI
BI (BPA-Bisimide)
BPA-BI
BPA-Bisimide
UI80-3

PURITY

Typical purity of 4,4'-BPA-BI is 94 wt%. Two isomers, 3,3'- and 3,4'-BPA-BI comprise approximately 6 wt% yielding a purity of all isomers > 99.9 wt%.

USE PATTERN

Bisphenol A Bisimide (BPA-BI) is a chemical intermediate that is primarily used as a reactive intermediate to make high molecular weight polyetherimide polymers.

FINAL TEST STATUS

Bisphenol A Bisimide **CAS RN: 54395-52-7**		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required
STUDY		**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**
PHYSICAL AND CHEMICAL DATA								
1.0	Melting Point	Y	Y	Y	N	N	Y	N
2.0	Boiling Point	Y	Y	Y	N	N	Y	N
3.0	Vapor Pressure	Y	N	N	Y	Y	Y	N
4.0	Partition Coefficient	Y	N	N	Y	Y	Y	N
5.0	Water Solubility	Y	Y	Y	N	N	Y	N
ENVIRONMENTAL FATE AND PATHWAY								
6.0	Photodegradation	Y	N	N	Y	Y	Y	N
7.0	Stability in Water	Y	N	N	Y	Y	Y	N
8.0	Transport and Distribution	Y	N	N	Y	Y	Y	N
9.0	Biodegradation	Y	Y	Y	N	N	Y	N
ECOTOXICITY								
10.0	Acute Toxicity to Fish	Y	N	N	N	Y	Y	N
11.0	Toxicity to Algae	Y	N	N	N	Y	Y	N
12.0	Acute Toxicity to Daphnia	Y	N	N	N	Y	Y	N
TOXICITY								
13.0	Acute Toxicity	Y	N	N	Y	N	Y	N
14.0	Genotoxicity *In Vitro or In Vivo* (Chromosome Aberration Test)	Y	Y	Y	N	N	Y	N
15.1	Genotoxicity *In Vitro* (Bacterial Test)	Y	N	N	Y	N	Y	N
15.2	Genotoxicity *In Vitro* (Mammalian Cells)	Y	Y	Y	N	N	Y	N
16.0	Repeated Dose Toxicity	Y	N	Y	N	N	Y	N
17.0	Reproductive Toxicity	Y	Y	Y	N	N	Y	N
18.0	Developmental Toxicity / Teratogenicity	Y	N	Y	Y	N	Y	N

ROBUST SUMMARY

PHYSICAL AND CHEMICAL DATA

1.0 MELTING POINT

Value:	139-150°C
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Sublimation:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 102 (1993)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4,4'-Bisphenol A bis-(N-methylphthalimide) [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]; Lot UI0058 from General Electric Company; Purity: 93.8%.
Remarks:	The melting point (MP) of a calibration substance (phenanthrene) was determined in duplicate. The MP of BPA-BI was determined in duplicate as follows: The sample was placed at the sealed end of a glass capillary tube to a height of approximately 2 mm. The tube was placed in the melting point apparatus, the heating block of which was heated to approximately 5 °C below the MP. The contents of the capillary were heated at 1 °C/min and the temperatures of Stages A (wet point), B (shrinkage point), C (collapsing point), D (liquefying point), and E (final melting stage) were recorded. It was also noted if the sample changed color or appeared to decompose during the test. The temperature range from Stage A-E is the melting point.
Results:	The calibration substance, phenanthrene, gave an average melting point of 97 - 99 °C, which, when compared to the expected MP of 99 - 101 °C, indicated a bias of 2 °C. The MP results for BPA-BI were adjusted 2 °C upward to account for this bias. The observed bias adjusted average MP of BPA-BI was 139-150 °C. The wide MP range was likely due to the presence of ~6% impurities that consisted of other regioisomers of BPA-BI. No color change was observed during the test.
Reference:	Reimer, G.J. (2004). Unpublished Report No. RAA7213 1448-MP (Project No. SP7213 1448-MP) entitled "Physical/Chemical Property of BPA-BI; CAS # 54395-52-7: Melting Point (OECD 102)", dated April 23, 2004 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

2.0 BOILING POINT

Value:	>315°C
Pressure:	102.2 kPa
Decomposition:	Yes [ ] No [X] Ambiguous [ ]
Method:	OECD Test Guideline 103 (1993)
GLP:	Yes [X] No [ ] ? [ ]

Test Substance:	4,4'-Bisphenol A bis-(N-methylphthalimide) [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]; Lot UI0058 from General Electric Company; Purity: 93.8%.
Remarks:	BPA-BI was placed in a Pyrex test tube (10 – 15 mL) along with a few boiling chips. The thermocouple tip was placed in the sample and connected to the digital logger. The tube and thermocouple were placed in aluminum block on an asbestos screen. A gas burner was used to heat the block and the fume hood door was closed. General observations were recorded, such as change in color, presence of smoke and if the material boiled. The burner was turned off when the substance boiled or the thermocouple reading was 310 °C. BPA-BI did not boil at any thermocouple temperature up to 310 °C. This method was calibrated by using ethylene carbonate as a reference substance. The observed duplicate boiling points of the Reference Substance, ethylene carbonate (EC), were 244 °C at 102.2 kPa. This compared to the reported BP of 249°C at 102.2 kPa, indicating an average deviation/bias of 5 °C. The duplicate BPA-BI heating curves did not show a distinct boiling plateau up to the maximum temperature of 310 °C. This was supported by the fact that BPA-BI was not observed to boil during the test. No BPA-BI color change was observed during the test. Therefore, the bias-corrected BP of BPA-BI was greater than 315 °C at 102.2 kPa. BP measurement at reduced pressure was not performed because the calculated BP at atmospheric pressure was greater than 300 °C, which is the regulatory limit set by the U.S. EPA (EPA, 1999) and Environment Canadian (CEPA, 1993).
Reference:	Reimer, G.J. (2004). Unpublished Report No. RAA7213 1448-BP (Project No. SP7213 1448-BP) entitled "Physical/Chemical Property of BPA-BI, CAS # 54395-52-7: Boiling Point (OECD 103)", dated April 23, 2004 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

3.0 VAPOR PRESSURE

Value:	7E-17 Pa at 25°C
Decomposition:	Yes [ ] No [ ] Ambiguous [ ] Not Applicable [X]
Method:	Calculated [X] Measured [ ]
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	4,4'-Bisphenol A bis-(N-methylphthalimide) [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]
Remarks:	Vapor pressure was calculated by computer software from Advanced Chemistry Development Inc. (ACD). The calculated VP of BPA-BI (7E-17 Pa at 25 °C) was less than the detection limit (~ 1E-5 Pa) of the most sensitive vapor pressure method (gas saturation method; OECD, 1995). The model is based on the boiling point at 760 mmHg. Boiling points determined at other pressures are recalculated using the Hass and Newton equations. The boiling point for BPA-BI was previously determined to be

	>315°C at 102.2 kPa (760 mmHg). The following equation is used to calculate the vapor pressure(Δ_{vap}):
	$\Delta_{vap}H(T_b) = [(\varphi*2.303*R*T_b(K))]*0.839594 + 3.9039.$
	ACD values show a higher correlation coefficient, when plotted against experimental values, compared to using the Watson method. Also, the ACD values show generally lower deviations from the experimental vapor pressures compared to the 'Watson values.' The OECD (OECD, 1995; Guideline 104) supports the use of the Watson correlation for the calculation of VP, but does not specifically reject other calculation methods. Using the ACD method, the vapor pressure for BPA-BI was determined to be 7E-17 Pa at 25 °C.
Reference:	Reimer, G.J. (2004). Unpublished Report No. RAA7213 1448-VP entitled "Physical/chemical property of BPA-BI; CAS # 54395-52-7: Vapor Pressure (OECD 104)", dated March 12, 2004 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 2) Valid with restrictions, calculated value.

4.0 PARTITION COEFFICIENT ($Log_{10}P_{ow}$)

Log Pow:	8.1 ± 0.5
Temperature:	24°C
Method:	Calculated [X] Measured [ ] Based on OECD Test Guideline 107 (1993)
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	4,4'-Bisphenol A bis-(N-methylphthalimide) [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]
Remarks:	The *n*-octanol/water partition coefficient (P_{ow}) was calculated using software from Advanced Chemistry Development Inc. (ACD). Based on the calculated $\log_{10} P_{ow}$ of the Test Substance 4,4'-bisphenol A bis-(N-methylphthalimide) (BPA-BI) of 8.1 ± 0.5 and a maximum allowable test concentration of 0.01 M in the OECD Guideline 107 "flask" method (OECD, 1993), the equilibrium concentration of BPA-BI in the aqueous phase of an *n*-octanol/aqueous 2-phase solution was calculated at 0.043 µg/L, which is more than 10-times less than the analytical method detection limit (DL) of 0.5 µg/L. Therefore, it was judged that BPA-BI would not be observed in the aqueous phases of a P_{ow} test at concentrations above the method DL, and that the result of such a test would not be a specific P_{ow} value, but, rather, a minimum P_{ow} value. In light of this, a calculated $\log_{10} P_{ow}$ of 8.1 ± 0.5 is reported for BPA-BI in lieu of laboratory results.

Reference:	Reimer, G.J. (2005). Unpublished Report No. RRA7213 1448-Pow entitled "Physical/chemical property of BPA-BI; CAS # 54395-52-7: n-Octanol/Water partition Coefficient (P_{ow}, OECD 107) dated July 8, 2005 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates, Inc., Vancouver, BC, Canada.
Reliability:	(Klimisch Code 1) Valid without restrictions.

5.0 WATER SOLUBILITY

5.1. SOLUBILITY

Value:	< 0.5 μg/L
Temperature:	24.0 °C
Description:	Miscible [ ]; Of very high solubility [ ]; Of high solubility [ ]; Soluble [ ]; Slightly soluble [ ]; Of low solubility [ ]; Of very low solubility [X]; Not soluble [ ]
Method:	OECD Test Guideline 105 (1995)
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	4,4'-Bisphenol A bis-(N-methylphthalimide) [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]; Lot U10058 from GE Plastics; Purity: 93.8%.
Remarks:	Stock Solution SkBI5: BPA-BI (10.17 mg) was dissolved in 10.00 mL of $CHCl_3$ in a volumetric flask resulting in a BPA-BI solution (1.017 mg/mL) in $CHCl_3$. Diluted solution D2 (37.8 μg/mL): SkBI5 (186 μL; glass syringe) was diluted in $CHCl_3$ to 5.00 mL in a volumetric flask. Aqueous Test Solutions: BPA-BI (10-11 mg) was ground if necessary to achieve an estimated particle size of < 0.1 mm and placed in glass centrifuge tubes followed by 27 mL of deionized water. There were also two blanks. The contents of all tubes were mixed (tumbled end-over-end; approx. 1 rev/3 sec) for 1.0 to 3.7 days. When the tubes were removed from the mixer, they were stored on the bench and the ambient room temperature was recorded. When the last tubes were removed from the mixer, presence of undissolved BPA-BI was noted and the pH was recorded in a blank and a BPA-BI solution tube. The tubes were centrifuged (5000 rpm, 20 min) and equilibrated for 2.5-3 days on the bench. The ambient temperature on the bench (24.0ºC) was recorded at the completion of equilibration. Each supernatant (including blank; approx. 17 mL) was combined (~34 mL) and filtered (0.45 μm filter) into 40 mL purge and trap vials. Two mL $CHCl_3$ was added to each vial and the mixtures were extracted along with the Calibration Solutions (as described below). Calibration Solutions: Five milliliters of $CHCl_3$ and 70 μL of solution D2 were combined in a glass tube and labeled DH5 (nominal BPA-BI of 0.0307 μg/mL in aqueous concentration). The Solution DH5 was serially diluted (2.00 mL) to yield calibration solutions ranging from 0.0307 to 0.0019 μg/mL BPA-BI. There was also a blank dilution (0.0 μg/mL BPA-BI). These solutions were transferred to 40 mL purge and trap vials

containing 34 mL of deionized water. The solutions were extracted as per below.

Recovery Standard: A second series of Calibration Extracts were prepared as described for the Calibration Solutions with the exception that 125 µL of D2 was added instead of 70 µL.

Extraction and Analyses: The $CHCl_3$/aqueous solutions (Saturated, Calibration and Recovery Solutions) were rapidly mixed end-over-end (~ 1 rev/s) for 10 minutes. The $CHCl_3$ layers were transferred to 1.8 mL glass crimp-top vials and analyzed by normal phase HPLC-UV using the following conditions: injection volume, 100 µL; column, Supelcosil HPLC-Si, 15 cm x 4.6 mm; column temp, ~ 22ºC; mobile phase, $CHCl_3$ 100%; run time, 6 min; column flow rate, 0.7 mL/min; and detector monitoring, 245 nm and 600 nm reference.

The concentrations (*C*, µg/mL) of BPA-BI in aqueous test solutions were calculated as follows: $C = (S-b) \div m$; where *S* was the area of the BPA-BI peak, *m* was the slope and *b* was the y-intercept of the calibration curve.

The recovery (R, %) of BPA-BI from water by the $CHCl_3$ extraction was calculated as follows: $R = (1/X) * 100$; where *X* was the observed/nominal BPA-BI concentration ratio for the Recovery Standard.

Results:

The calibration curve from the Calibration Solutions showed a linear relationship with a correlation coefficient of 0.998, indicating acceptable method precision for the analysis of chloroform extracts of water spiked with BPA-BI. The recovery of BPA-BI from spiked water by the chloroform extraction procedure was 85%. Recovery was accounted for in the quantification of BPA-BI in aqueous test solutions because HPLC calibration was achieved by analyzing spiked aqueous Calibration Solutions.

The detection limit was defined as the lowest concentration of an analyte that an analytical process can reliably detect. The Detection Limit (DL) was estimated from the HPLC chromatograms of Calibration Extracts as the lowest BPA-BI concentration in spiked water that would give a peak which is reliably distinguishable form the baseline noise. The estimated DL was 0.0005 µg/mL (0.5 µg/L). The quantification limit (QL, 0.0024 µg/mL = 2.4 µg/L) was estimated as 5-times the DL according to Vial and Jardy (1999).

After mixing for up to 3.7 days, undissolved BPA-BI was observed in all aqueous test solutions, as is required for saturation. There was no measurable difference in the pH of the BPA-BI aqueous test solutions as compared to the pH of the blank. When analyzed by HPLC-UV, BPA-BI was detected in the aqueous test solutions mixed for one day at 0.001 µg/mL, which was less than the QL of 0.0024 µg/mL. Only traces of BPA-BI were detected in the aqueous test solutions mixed for 2.0 and 3.7 days. The higher BPA-BI concentrations found in the aqueous test solutions mixed for 1 day may have been due to the passage of a minor amount of undissolved suspended BPA-BI during filtration. It was concluded that the water solubility of BPA-BI was less than 0.0005 µg/mL at 24.0ºC.

Reference:	Reimer, G.J. (2005). Unpublished Report No. RAA7213 1448-WS entitled "Physical/chemical property of BPA-BI; CAS # 54395-52-7: Water Solubility (OECD 105)", dated July 8, 2005 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada. Vial, J. and Jardy, A.. (1999). Experimental Comparison of the Different Approaches to estimate LOD and LOQ of an GC-FID method. Anal. Chem. 71, 2672-2677.
Reliability:	(Klimisch Code 1) Valid without restrictions.

5.2. pH VALUE, pKa VALUE

No studies were found.

ENVIRONMENTAL FATE AND PATHWAYS

6.0 PHOTODEGRADATION

Method:	Calculated [X] Measured [ ]
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	1H-Isoindole-1,3(2H)-dione, 5,5'- (1-methylethylidene)bis(4,1-phenyleneoxy) bis 2-methyl- [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]
Concentration:	Not applicable
Temperature °C:	Not applicable
Direct photolysis:	Not applicable
Indirect photolysis:	Not applicable
Breakdown products:	Not applicable
Value:	Overall OH Rate Constant (k_{phot}) =47.0366 E-12 cm3/molecule-sec; Half-life ($t_{1/2}$) = 2.729 hrs (12-hr day; 1.5E6 OH/cm3)
Remarks:	Atmospheric photo-oxidation potential, mediated by reaction with hydroxyl radicals, was estimated using the submodel, Atmospheric Oxidation Potential for Windows (AOPWIN v.1.91, EPIWIN v3.11, U.S. EPA, 2003). The SAR methods rely on structural features of the subject molecule. The model calculates a second-order half-life with units of cm^3/molecules-sec. A pseudo-first order photo-degradation rate is in turn based on the second order rate of reaction (cm^3/molecules-sec) with hydroxyl radicals (HO•), assuming first-order kinetics and an HO• concentration of 1.5 E+06 molecules/cm^3 and 12 hours of daylight. Pseudo-first order half-lives ($t_{1/2}$) were then calculated as follows: $t_{1/2} = 0.693 / [k_{phot} \times HO\bullet \times 12\text{-hr} / 24\text{-hr}]$.

The EPIWIN model (v 3.11) was run using the following measured physical chemical properties:

Water solubility = 0005 mg/L;
Vapor pressure = 5E-019 mm Hg;
Boiling point = 315°C; and
Melting point 145°C.

Reference: U.S. EPA (U.S. Environmental Protection Agency). 2003. EPI Suite, Version 3.11; AOPWIN Program, Version 1.91; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

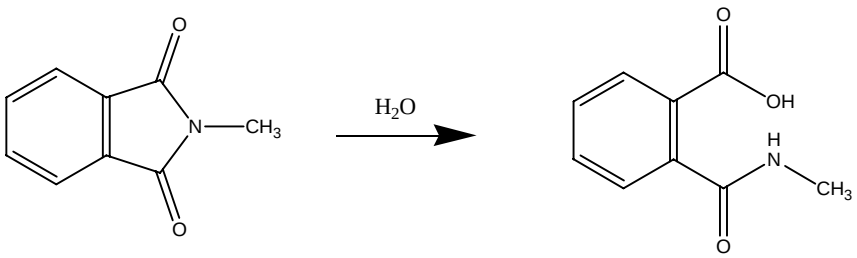
Reliability: (Klimisch Code 2) Valid with restrictions, calculated value.

7.0 STABILITY IN WATER

Type: Abiotic (hydrolysis) [X]; biotic (sediment) [ ]
Half life: 1 yr, < 1 yr and < 1 yr at pH 4, pH 7, and pH 9 respectively
Degradation: Yes (see remarks below)
Method: Expert Statement based on OECD Test Guideline 111 (2004)
GLP: Yes [ ] No [X] ? [ ]
Test Substance: 4,4'-Bisphenol A bis-(N-methylphthalimide)
[Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]
Remarks: The hydrolysis of BPA-BI testing in accordance with OECD Guideline 111 was precluded due to: the relatively high co-solvent concentrations required to dissolve BPA-BI in aqueous buffer solutions due to its low water solubility and that it was not feasible within a normal laboratory effort to determine the effect of the cosolvent on the hydrolysis reaction.

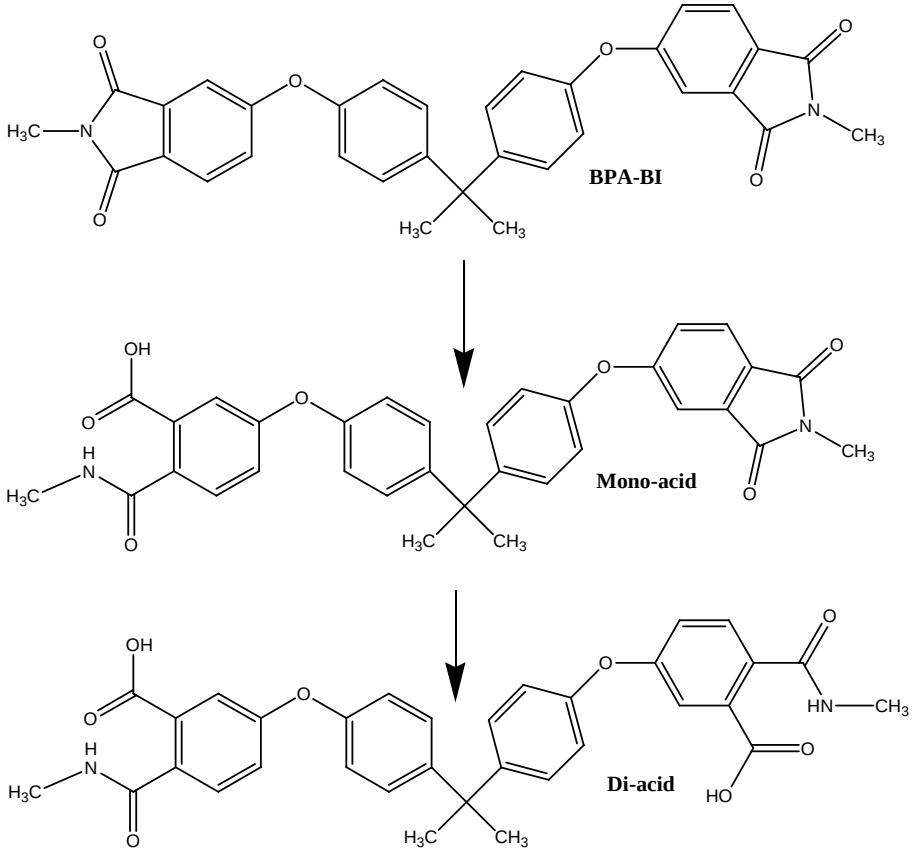
In the absence of being able to analyze BPA-BI in accordance with OECD 111, the hydrolytic properties of BPA-BI were postulated on the basis of 'alternative' preliminary experimental results and surrogate data on N-methylphthalimide (PI), a structural analog.

The hydrolysis of PI results in the phthalimide ring opening to give the corresponding phthalamic acid hydrolysis product as shown below.



Hydrolysis of N-methylphthalimide (PI)

As BPA-BI contains the same hydrolytically susceptible group, it was concluded that BPA-BI would hydrolyze in the same manner to produce the mono- and di-acid products.



Hydrolysis reactions of BPA-BI (One of two possible regioisomers is shown for the mono-acid. Similarly, the di-acid shown is one of 4 possible regioisomers.)

Results:

Experimental Results at pH 9: The hydrolysis half-life of BPA-BI in pH 9 buffer/acetonitrile (50/50 v/v) was experimentally estimated at 1.3 h at 22°C. Based on this half-life, and the expectation that the hydrolysis rate would be faster in pure pH 9 buffer compared to buffer/acetonitrile (50/50 v/v) due to the higher concentration of water in the former, it was concluded that BPA-BI would be characterized as hydrolytically unstable under these conditions in accordance with the criteria outlined in OECD Guideline 111 (i.e. half-life < 1 yr at 25 °C).

The observed HPLC peak patterns were consistent with the expected hydrolysis reaction pathway of phthalimide ring opening to give the N-methylphthalamic acid hydrolysis products.

Surrogate Results at pH 4 and pH 7: Unlike the facile hydrolysis of BPA-BI in pH 9 buffer/acetonitrile (50/50 v/v) at 22 °C, BPA-BI was found to be relatively stable under similar conditions of time and temperature at pH 4 and pH 7. This was consistent with the general observation that the hydrolysis of amides is faster under basic conditions compared to acidic and neutral conditions. Therefore, due to the low solubility of BPA-BI

and the need for high cosolvent concentration, experimental results on the hydrolysis of BPA-BI at pH 4 and pH 7 did not provide useful information regarding hydrolytic stability, and, therefore, surrogate data are presented instead.

The hydrolytic properties of N-methylphthalimide (PI), which is structurally similar to BPA-BI at the site of hydrolysis, were experimentally determined (Reimer, 2001) in accordance with OECD Guideline 111. The above (pH 9) BPA-BI hydrolysis half-life of 1.3 h at 22 °C was consistent with the observed PI half-life of 2.2 h at pH 9 and 25 °C, in the absence of cosolvent. These results supported the validity of PI as a surrogate compound for the assessment of the hydrolytic stability of BPA-BI.

PI was also reported (Reimer, 2001) to be hydrolytically unstable in pH 7 buffer (half-life = 88 h at 25 °C). Based on this half-life, it was concluded that BPA-BI would also be hydrolytically unstable at pH 7 according to the OECD Guideline 111 criteria.

On the other hand, the reported (Reimer, 2001) half-life of PI in pH 4 buffer was considerably longer at 1.16 yr at 25 °C, indicating that PI was "borderline stable" according to the OECD Guideline 111 criteria. The Hammett structure-activity relationship suggested that the half-life of BPA-BI, under a given set of conditions, would be slightly less than that of PI. This would be due to the presence of an electron-withdrawing aryloxy (ether) group attached to the aromatic phthalimide ring of BPA-BI, which is absent in PI. Therefore, it could only be concluded that the hydrolysis half-life of BPA-BI at 25 °C was approximately 1 yr at pH 4, and that its hydrolytic stability at pH 4 was a borderline case for "hydrolytically stable", with a more definitive determination not possible on the basis of the surrogate data as presented for PI.

Based on experimental and surrogate data as summarized, it was concluded that BPA-BI was hydrolytically unstable at pH 7 and pH 9, with half-lives of less than 1 yr at 25 °C (OECD, 2004). The half-life of BPA-BI at pH 4 was estimated at approximately 1 yr at 25 °C: A more definitive assessment of hydrolytic stability at pH 4 was not possible.

In applying these stabilities to issues related to the environmental impact and/or toxicity of BPA-BI, the relatively low water solubility of the Test Substance (< 0.5 µg/L at 24 °C) should be considered.

Reference: Reimer, G.J. (2005). Unpublished Report No. RAA7213 1448-Hy entitled "Physical/chemical property of BPA-BI; CAS # 54395-52-7: Hydrolytic Stability (OECD 111)", dated July 3, 2005 for General Electric Company, Pittsfield, MA, USA; from Reimer Analytical & Associates, Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

8.0 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS, INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

8.1 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

Type:	Fugacity model Level III
Media:	Other: air, water, soil, sediment
Method:	Calculated [X] Measured [ ]
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	1H-Isoindole-1,3(2H)-dione, 5,5'- (1-methylethylidene)bis(4,1-phenyleneoxy) bis 2-methyl- [Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]
Value:	Air < 0.1% Water = 1.25% Soil = 34.7% Sediment = 64%

Remarks:	Default values were assumed for environmental compartment descriptions, dimensions, and properties, advective and dispersive properties. Chemical-specific input parameters were:
	molecular weight = 546.58 g/mol;
	vapor pressure = 5E-019 mm Hg;
	melting point = 145°C;
	aqueous solubility = 0.0005 mg/L; and
	boiling point = 315ºC.
	Emissions were assumed to be equally to air, water and soil.
	
	Air: $t_{1/2}$ = 5.64 hr; emissions = 1000 kg/hr
	Water: $t_{1/2}$ = 3600 hr; emissions = 1000 kg/hr
	Soil: $t_{1/2}$ = 3600 hr; emissions = 1000 kg/hr
	Sediment: $t_{1/2}$ = 14400 hr; emissions = 0 kg/hr

Reference:	U.S. EPA (U.S. Environmental Protection Agency). 2003. EPI Suite, Version 3.11; Level III Fugacity Model; PC-Computer software developed by EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).
Reliability:	(Klimisch Code 2) Valid with restrictions, calculated value.

9.0 BIODEGRADATION

Type:	Aerobic [X]; Anaerobic [ ]
Inoculum:	Adapted [ ]; Non-adapted [X]
Concentration of the chemical:	10 mg/L Related to COD [ ]; DOC [ ]; Test substance [X]; or, 4.0 mg/L as ThOD
Medium:	Water [ ]; Water-sediment [ ]; Soil [ ]; Sewage treatment [ ]; Other [X]: Deionized water with nutrients

Contact time: 28 days at 20.2 ± 1°C
Degradation: 0.9%
Results: Readily biodeg. [ ]; Inherently biodeg. [ ]; Other [ ];
Under test condition, no (significant) biodegradation observed [X]
Kinetic of test substance:

Mean % Biodegradation

Day	% Degradation of BPA-BI	% Degradation of Reference Substance	% Degradation of Toxicity Control
0	0.0	0.0	0.0
4	1.8	---	---
7	0.6	74.1	74.3
11	1.2	---	---
14	1.2	82.2	79.6
18	0.4	---	---
21	0.3	76.6	67.2
24	0.3	---	---
28	0.9	81.9	73.1

The results of this study indicated that the test substance was not readily biodegradable. Biodegradation did not reach >60% in a 10-day window within the 28 day test period. On Day 28, there was 0.9% biodegradation in the test bottles. In addition, there was no significant difference in biodegradation between the toxicity control (containing test substance and reference substance) and the reference substance alone. On Day 14, there was 80% biodegradation in the toxicity control and 82% in the reference substance alone. Therefore, it is unlikely that the low biodegradation observed was caused by toxicity of the test substance to the microbes.

Reference substance: Sodium benzoate
Toxicity control: BPA-BI and Sodium benzoate
Kinetic of control substance: See Above
Degradation Products: Yes [ ] No [ ] Not measured [X]
Method (Year): OECD Test Guideline 301D (1992)
GLP: Yes [X] No [ ] ? [ ]
Test Substance: 4,4'-Bisphenol A bis-(N-methylphthalimide)
[Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7]; Lot UI0058 from General Electric Company; Purity: >99.9%.

Remarks: Following is a summary of test conditions

Parameter	Test Condition
Test type	OECD 301D – Closed Bottle Test (non-renewal) Aerobic
Duration	28 days
Inoculum	Secondary effluent from Lulu Island Domestic Wastewater Treatment plant, Richmond, BC
Temperature	20.2 $\pm$ 1°C
O_2 Determination Method	Dissolved oxygen concentration using an oxygen electrode
Nitrification Determination Method	Not determined based on results
Test vessel	300-mL BOD bottles
Test volume	300 mL
Replicates	Two
Aeration	None
Controls	1. Seeded control (inoculated blank) 2. Positive control (Reference substance plus inoculum) 3. Toxicity control (Reference substance, BPA-BI and inoculum)
Nominal BPA-BI concentration	10 mg/L
Nominal Reference Substance concentration	2 mg/L
Criterion for Ready Biodegradability	>60% degradation in 10-day window within 28 day test period

Mean Biological Oxygen Demand (mg O_2/mg test substance)

Day	% Degradation of BPA-BI	% Degradation of Reference Substance	% Degradation of Toxicity Control
0	0.0	0.0	0.0
4	0.04	---	---
7	0.01	1.24	1.24
11	0.02	---	---
14	0.02	1.37	1.33
18	0.01	---	---
21	0.01	1.28	1.12
25	0.01	---	---
28	0.02	1.37	1.22

Reference: Serben, K. (2005). Unpublished Report No. TOX0449 entitled "Closed Bottle Test of 4,4'-Bisphenol A Bis-(N-Methylphthalimide)(CAS RN: 54395-52-7)(OECD Guideline 301D)", dated January 28, 2005 for General Electric Company, Pittsfield, MA, USA; from Vizon SciTec Inc., Vancouver, BC, Canada.

Reliability: (Klimisch Code 1) Valid without restrictions.

ECOTOXICOLOGICAL DATA

10.0 ACUTE/PROLONGED TOXICITY TO FISH

Due to the low water solubility of BPA-BI (<0.5 µg/L at 24.0ºC), aquatic toxicity studies were not conducted.

11.0 TOXICITY TO AQUATIC PLANTS (E.G. ALGAE)

Due to the low water solubility of BPA-BI (<0.5 µg/L at 24.0ºC), aquatic toxicity studies were not conducted.

12.0 ACUTE TOXICITY TO AQUATIC INVERTEBRATES (E.G. DAPHNIA)

Due to the low water solubility of BPA-BI (<0.5 µg/L at 24.0ºC), aquatic toxicity studies were not conducted.

TOXICITY

13.0 ACUTE TOXICITY

13.1 ACUTE ORAL TOXICITY

Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [ ] LD_{L0} [ ] Other [X]
Species/Strain:	Rat/Spartan strain
Sex:	Male
# Animals:	4
Vehicle:	Corn oil
Value:	Not applicable
Method:	Not specified
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	AR No. 82895 (Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7); from General Electric Company; Purity: See "Chemical Identity and Use Information" section.
Remarks:	The test material was administered to two rats each at doses of 500 and 5000 mg/kg. The rats weighed from 206 to 236 g at the initiation of the study. Animals were fasted from food overnight prior to dosing. The test compound was suspended in corn oil at concentrations enabling the administration of 10 mL/kg for the 500 mg/kg dose and 20 mL/kg for the 5000 mg/kg dose. All rats were observed for mortality for a period of 14 days. Body weights were measured initially and at day 14.
Results:	All rats survived and gained weight during the 14-day post-dosing period.
Reference	Wazeter, F. X. and Goldenthal, E.I. (1974). Unpublished report for Project No. 313-034 entitled "Acute Toxicity Screening Studies in Rats and Rabbits", dated May 16, 1974 for General Electric Company, from International Research and Development Corporation, Mattawan, MI, USA.

Reliability:	(Klimisch Code 2) Valid with restrictions. Only four animals dosed, two per group.

13.2 ACUTE INHALATION TOXICITY

No studies were found.

13.3 ACUTE DERMAL TOXICITY

Type:	LD_0 [ ] LD_{100} [ ] LD_{50} [ ] LD_{L0} [ ] Other [X]
Species/Strain:	Rabbit/New Zealand White
Value:	Not applicable
Method:	Not specified
GLP:	Yes [ ] No [X] ? [ ]
Test Substance:	AR No. 82895 (Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7); from General Electric Company; Purity: See "Chemical Identity and Use Information" section.
Remarks:	Two New Zealand White rabbits (one male and one female) were used at each of two doses. The rabbits weighed from 2432 to 2747 grams at study initiation. Body weights were measured initially and at 14 days. The compound was applied to the clipped back of each rabbit. Two rabbits received 200 mg of the test substance/kg body weight and two rabbits received 2000 mg of the test substance/kg body weight. The application area was wrapped with a gauze bandage and occluded with plastic wrap. After 24 hours, the bandages were removed and the application areas were washed with tepid tap water. The rabbits were observed for mortality for a period of 14 days.
Results:	All rabbits survived the 14-day observation period. Three of the rabbits exhibited body weight gains and one rabbit showed a slight (151 gram) loss in body weight during the 14-day observation period.
Reference:	Wazeter, F.X. and Goldenthal, E.I. (1974). Unpublished report for Project No. 313-034 entitled "Acute Toxicity Screening Studies in Rats and Rabbits", dated May 16, 1974 for General Electric Company, from International Research and Development Corporation, Mattawan, MI, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Only two rabbits exposed per group.

14.0 GENETIC TOXICITY *IN VITRO* OR *IN VIVO* (CHROMOSOMAL ABERRATIONS)

Type:	*In vitro* mammalian chromosome aberration test
System of testing:	Chinese hamster ovary (CHO) cells
Concentration:	0, 6.25, 12.5, 25, 35, 50, 75, 100 μg/mL (4-hr treatment w/o S9 mix) 0, 6.25, 12.5, 25, 35, 50, 75, 100, 125 μg/mL (20-hr treatment w/o S9 mix) 0, 6.25, 12.5, 25, 50, 75 μg/mL (4-hr treatment with S9 mix)
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative with and without metabolic activation
Cytotoxicity conc:	With metabolic activation: None

	Without metabolic activation:	≥ 450 μg/mL (4 hr exposure)		
	Without metabolic activation:	≥ 150 μg/mL (20 hr exposure)		
Precipitation conc:	With metabolic activation:	≥ 75 μg/mL		
	Without metabolic activation:	≥ 75 μg/mL		
Genotoxic effects:		+	?	-
	With metabolic activation:	[ ]	[ ]	[X]
	Without metabolic activation:	[ ]	[ ]	[X]

Method: OECD Test Guideline 473 (1998)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: Bisphenol A-Bisimide (BPA-BI; CAS RN 54395-52-7); from General Electric Plastics; Purity: >99.9%.

Remarks: Description of test procedure: A preliminary toxicity assay was performed for the purpose of selecting doses for the chromosome aberration assay and consisted of an evaluation of test article effect on cell growth. CHO cells were seeded for each treatment condition at approximately $5x10^5$ cells/25 cm^2 flask and were incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for 16-24 hours. Treatment was carried out by refeeding the flasks with complete medium for the non-activated study or S9 reaction mixture for the activated study, to which was added 50 μL dosing solution of test article in solvent or solvent alone. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. The cells were treated for 4 hours with and without S9, and continuously for 20 hours without S9. At completion of the 4-hour exposure period, the cells were washed refed with complete medium and returned to the incubator for a total of 20 hours from the initiation of treatment. At 20 hours after the initiation of treatment the cells were harvested. The presence of test article precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control. In the preliminary toxicity assay, the maximum dose tested was 1500 μg/mL. The test article was soluble in treatment medium at ≤ 15 μg/mL and precipitate was observed at ≥ 45 μg/mL. The osmolality of the test article concentrations in treatment medium were acceptable because they did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was approximately 7.5. Based on the toxicity study, the doses chosen for the chromosome aberration assay were 0, 6.25, 12.5, 25, 35, 50, 75 and 100 μg/mL (4-hr treatment w/o S9 mix), 0, 6.25, 12.5, 25, 35, 50, 75, 100 and 125 μg/mL (20-hr treatment w/o S9 mix), and 0, 6.25, 12.5, 25, 50 and 75 μg/mL (4-hr treatment with S9 mix). Samples were run in duplicate, with and without metabolic activation.

Selection of doses for microscopic analysis: The first criterion, specified by the Guideline, is to select the highest dose with at least

50% reduction in cell growth or mitotic index relative to the solvent control with a sufficient number of scorable metaphase cells, regardless of test article precipitation in the treatment medium. For this study, the mitotic index was used to select the highest dose for each test condition. Two lower doses also were included.

For the chromosome aberration assay, CHO cells were seeded and treated as described above. The osmolality in treatment medium of the solvent and of the highest test article concentration, the lowest precipitating test article concentration and the highest soluble test article concentration were measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape. A concurrent toxicity test was conducted for each treatment. After cell harvest the cells were counted, test article precipitate was assessed and cell viability was determined by trypan blue dye exclusion. Cell counts and viability were used to determine cell growth inhibition.

The cells were exposed to the test article continuously for 4 or 20 hours in the non-activated study, and for 4 hours in the activated study. After the exposure period for the 4-hour exposure groups, the cells were washed and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition. Two hours after the addition of Colcemid®, metaphase cells were harvested. Cells were collected approximately 20 hours after initiation of treatment. Slides were prepared from cells of each treatment and the cells stained with 5% Giemsa.

Evaluation of metaphase cells: The percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Initially, the non-activated and S9 activated 4-hour exposure groups were evaluated for chromosome aberrations and since a negative result was obtained in the non-activated 4-hour exposure group, the non-activated 20-hour continuous exposure group was then also evaluated for chromosome aberrations. When possible, a minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations. The number of metaphase spreads that were examined and scored per duplicate flask may have been reduced if the percentage of aberrant cells reached a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) were also recorded. Chromatid and

isochromatid gaps were recorded but not included in the analysis. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored.

Evaluation of test results: The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell were calculated and reported for each group. Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose group, the Cochran-Armitage test was used to measure dose-responsiveness. As a guide to interpretation of the data, the test article was considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historic solvent controls may be judged as not biologically significant. Test articles not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Criteria for a Valid Test: The frequency of cells with structural chromosome aberrations in the solvent control must be within the range of the historical solvent control. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.
Negative and Positive controls: Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 μg/mL. Cyclophosphamide (CP) was used as the positive control in the S9 activated study at final concentrations of 10 and 20 μg/mL. The solvent vehicle for the test article, dimethyl sulfoxide (DMSO), was used as the solvent control at the same concentration as that found in the test article-treated groups.

Results: In the chromosome aberration assay, the test article was soluble in treatment medium at doses ≤ 50 μg/mL and precipitate was observed at ≥ 75 μg/mL. The osmolality in the treatment medium of the highest concentration tested (125 μg/mL), was 401 mmol/kg. The osmolality in the treatment medium of the lowest precipitating concentration (75 μg/mL), was 397 mmol/kg. The osmolality in the treatment medium

of the highest soluble concentration (50 μg/mL), was 394 mmol/kg. The osmolality of the solvent (DMSO) in treatment medium was 394 mmol/kg. The pH of the highest concentration of test article in treatment medium was approximately 7.0.

4-hour harvest without metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 100 μg/mL, was 52% reduced relative to the solvent control. The doses selected for microscopic analysis were 25, 50 and 100 μg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (16.0%) was statistically significant.

4-hour harvest with metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 75 μg/mL, was 52% reduced relative to the solvent control. The doses selected for microscopic analysis were 12.5, 25, and 75 μg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the CP (positive control) treatment group (18.0%) was statistically significant.

20-hour harvest without metabolic activation: No toxicity of BPA-BI was observed. The mitotic index at the highest dose evaluated for chromosome aberrations, 50 μg/mL, was 51% reduced relative to the solvent control. The doses selected for microscopic analysis were 12.5, 25 and 50 μg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated groups was not significantly increased above that of the solvent control at any dose ($p \leq 0.05$, Fisher's exact test). The percentage of structurally damaged cells in the MMC (positive control) treatment group (25.0%) was statistically significant.

Summary of Test Results

Treatment (µg/mL)	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean ± SD)	Cells with Numerical Aberrations (%)	Cells with Structural Aberrations (%)
Vehicle (DMSO)	-	4	7.9	200	0.005 ± 0.071	3.0	0.5
Bisphenol A Bisimide (BPA-BI)							
25	-	4	7.5	200	0.010 ± 0.100	4.0	1.0
50	-	4	5.7	200	0.015 ± 0.122	3.5	1.5
100	-	4	3.8	200	0.015 ± 0.122	3.5	1.5
Positive control (MMC) 0.2	-	4	9.2	200	0.18 ± 0.434	2.5	16.0**
Vehicle (DMSO)	+	4	7.5	200	0.000 ± 0.000	3.5	0.0
Bisphenol A Bisimide (BPA-BI)							
12.5	+	4	7.6	200	0.010 ± 0.100	5.5	1.0
25	+	4	6.8	200	0.000 ± 0.000	4.5	0.0
75	+	4	3.6	200	0.010 ± 0.100	6.0	1.0
Positive control (CP) 10	+	4	5.7	100 [a]	0.300 ± 1.087	4.0	19.0**
Vehicle (DMSO)	-	20	9.1	200	0.000 ± 0.000	1.5	0.0
Bisphenol A Bisimide (BPA-BI)							
12.5	-	20	8.1	200	0.000 ± 0.000	1.5	0.0
25	-	20	8.5	200	0.010 ± 0.100	2.5	1.0
50	-	20	4.5	200	0.005 ± 0.071	1.5	0.5
Positive control (MMC) 0.1	-	20	8.1	100[a]	0.280 ± 0.533	2.5	25.0**

Treatment: Cells from all treatment regimens were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: **, $p \leq 0.01$; using the Fisher's exact test.
[a] Numerical aberrations are out of 200 cells scored.

Conclusion: The positive and solvent controls fulfilled the requirements for a valid test. Under the conditions of the assay, BPA-BI was concluded to be negative for the induction of structural and numerical chromosome aberrations in CHO cells in the presence and absence of S9 activation.

Reference: Gudi, R. and Rao, M. (2005). Unpublished Report No. AA79BU.331.BTL entitled "*In vitro* mammalian chromosome aberration test", dated April 27, 2005 for General Electric Company, Pittsfield, MA, USA; from BioReliance Corp., Rockville, MD, USA.

Reliability: (Klimisch Code 1) Valid without restrictions.

15.0 GENETIC TOXICITY *IN VITRO*

15.1 BACTERIAL TEST

15.1.1

Type:	Bacterial reverse mutation assay (Ames test)
System of testing:	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538
Concentrations:	0, 1, 10, 100, 500, 1000, 2500, 5000 and 10000 µg/plate
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative
Cytotoxicity conc.:	No cytotoxicity observed with and without metabolic activation:
Precipitation conc.:	Not stated
Genotoxic effects:	With metabolic activation: positive [ ]; ambiguous [ ]; Negative [X] Without metabolic activation: positive [ ]; ambiguous [ ]; Negative [X]
Method:	Ames et al. (1975) Mutation Research 31:347-365 Description of test procedure: The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 0.1 mL to 0.2 mL of the test organisms were treated with the test substance in the presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for approximately 48 hours at 37 ºC, and scored for the number of colonies growing on each plate. *Solvent and Positive controls:* Deionized water was the solvent for the test substance and served as the solvent control. For the non-activation assay, the following positive control substances were used: Sodium azide (for strains TA1535 and TA100); 2-Nitrofluorene (for strains TA1538 and TA98); and 9-Aminoacridine (for strain TA1537). The positive control substance, 2-anthramine was used for all tester strains with metabolic activation. Criteria for evaluating results: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, a test substance producing a positive response equal to three times the solvent control value is considered mutagenic. For strains TA98 and TA100, a test substance producing a positive response equal to twice the solvent control value is considered mutagenic. In addition, a positive response must be repeated in a separate assay. Activation system: S9 liver homogenate prepared from Aroclor 1254-induced Sprague-Dawley male rats. The S9 mix was prepared fresh each day of testing.

Year: 1981
GLP: Yes [ ] No [X] ? [ ]
Test substance: 02-81-011535-014 AR #93479 (Bisphenol A Bisimide; BPA-BI; CAS RN 54395-52-7); Purity: See "Chemical Identity and Use Information" section.
Results: The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains, was reported to be less than or approximately equal to the number of revertants in the solvent-treated negative control group, with and without metabolic activation.

Revertants Per Plate
Activation: None

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (distilled water)	14	5	16	36	123
Solvent (distilled water)	16	9	19	56	128
1	6	17	15	55	151
10	16	7	23	50	113
100	16	10	18	51	138
500	17	10	22	53	122
1000	15	8	23	61	170
2500	18	13	12	47	160
5000	19	19	15	63	126
10000	10	8	14	61	137
Positive Control	584	190	820	876	1143
Positive Control	645	299	925	913	1249

Revertants Per Plate
Activation: Rat Liver S9

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100
Solvent (distilled water)	11	11	24	69	141
Solvent (distilled water)	12	18	27	73	143
1	17	11	20	66	132
10	11	10	23	62	157
100	11	19	24	61	155
500	9	10	17	69	153
1000	15	7	18	62	161
2500	13	8	13	47	188
5000	12	12	23	65	172
10000	12	9	12	66	162
Positive Control	443	227	1555	1510	1608
Positive Control	450	308	1756	1554	1661

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference:	Jagannath, D. R. and Brusick, D. J. (1981), Unpublished report for Project No. 20988 entitled "Mutagenicity Evaluation of 02-81-011535-014 AR #93479", dated April 1981 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.
15.1.2	
Type:	Bacterial reverse mutation assay (Ames test)
System of testing:	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538; *Saccharomyces cerevisiae* strain D4
Concentrations:	0, 0.1, 1.0, 10, 100 and 500 μg/plate (TA98, TA100, TA1535, TA1537, TA1538, and D4); and 500, 1000, 2000 μg/plate (TA100) – second test
Metabolic activation:	With [ ]; Without [ ]; With and Without [X]; No data [ ]
Results:	Negative
Cytotoxicity conc.:	With metabolic activation: not stated Without metabolic activation: not stated
Precipitation conc.:	None
Genotoxic effects:	With metabolic activation: positive [ ]; ambiguous [ ]; Negative [X] Without metabolic activation: positive [ ]; ambiguous [ ]; Negative [X]
Method:	Ames et al. (1975) Mutation Research 31:347-365 Description of test procedure: The plate test consisted of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Approximately 10^8 cells were treated with the test substance in the presence and absence of a metabolic activation system (Aroclor 1254-treated rat liver supernatant). One plate was used per concentration. The plates were incubated for 48 hours at 37 ºC, and scored for the number of colonies growing on each plate. *Solvent and Positive controls:* Dimethylsulfoxide (DMSO) was the solvent for the test substance and served as the solvent control. For the non-activation assay, the following positive control substances were used: Methylnitrosoguanidine (for strains TA1535, TA100 and D4); 2-Nitrofluorene (for strains TA1538 and TA98); and quinacrine mustard (for strain TA1537). The positive control substances, 2-anthramine (strains TA1535 and TA100), 2-acetylaminofluorene (strains TA1538 and TA98) and 8-aminoquinoline (strain TA1537) were used with metabolic activation. The positive control substance used for D4 without activation was not identified in the report. Criteria for evaluating results: The solvent control values must be within the normal historical control range and the presence of a dose response is required for establishing mutagenicity. For strains TA1535, TA1537 and TA1538, if the solvent control value is within the normal range, a test

substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control is considered mutagenic. For strains TA98, TA100 and D4, a test substance producing a positive response over three concentrations with the lowest increase equal to twice the solvent control (TA100) or two to three times the solvent control (TA98 and D4) is considered mutagenic. In addition, a positive response must be repeated in a separate assay.

Activation system: S9 liver homogenate prepared from Aroclor 1254-induced male Sprague-Dawley rats.

Year: 1977

GLP: Yes [ ] No [X] ? [ ]

Test substance: 2,2-Bis[4-(3,4-dicarboxyphenoxy)phenyl]propane-bis-N-methyl imide (Bisphenol A Bisimide; BPA-BI; 09-77-011154-022); Purity: See "Chemical Identity and Use Information" section.

Results: The number of revertants/plate produced by treatment of the bacteria with the test substance at all concentrations and in all tester strains, except TA100 in the first test, was reported to be less than or approximately equal to the number of revertants in the solvent-treated negative control group, with and without metabolic activation. Doses of 1000 and 2000 μg/plate were used with TA-100 and the test was repeated because of an increased mutation frequency observed at 500 μg/plate. The results of the second test were negative.

Revertants Per Plate
Activation: None

Dose (μg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4*
Solvent (DMSO)	10	18	25	31	169 235	32
0.1	10	22	20	28	187 ---	46
1.0	16	15	19	33	216 ---	37
10	14	25	19	28	257 ---	33
100	12	18	21	27	216 ---	39
500	9	15	10	31	304 268	41
1000	--	--	--	--	--- 213	--
2000	--	--	--	--	--- 217	--
Positive Control	653	564	>1000	>1000	>1000	573

Revertants Per Plate
Activation: Rat Liver S9

Dose (µg/plate)	TA1535	TA1537	TA1538	TA98	TA100	D4*
Solvent (DMSO)	17	13	24	35	267 205	23
0.1	15	13	20	48	220 ---	
1.0	10	12	24	34	242 ---	20
10	12	27	29	36	228 ---	20
100	5	10	17	27	264 ---	19
500	11	14	24	39	316 210	22
1000	--	--	--	--	--- 246	21
2000	--	--	--	--	--- 269	--
Positive Control	131	213	574	891	831	48--

Conclusion: The test substance did not exhibit mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions according to the evaluation criteria.

Reference: Jagannath, D.R. and Brusick, D.J. (1977). Unpublished report for Project No. 20838 entitled "Mutagenicity evaluation of 09-77-011154-022", dated November 1977 for General Electric, Schenectady, NY, USA; from Litton Bionetics, Inc., Kensington, MD, USA.

Reliability: (Klimisch Code 2) Valid with restrictions. Acceptable study report that meets basic scientific principles.

15.2 NON-BACTERIAL *IN VITRO* TEST (MAMMALIAN CELLS)

Type: *In vitro* mammalian cell gene mutation test (Mouse lymphoma assay)

System of testing: Mouse lymphoma L5178Y cells

Concentration: Preliminary Toxicity Assay: 0, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500 and 1500 µg/mL 4-hr with/without activation and 24-hr without activation
Initial Assay: 0, 10, 25, 50, 75 and 100 µg/mL with and without activation (4-hour exposure)
Extended Assay: 0, 5, 10, 25, 50, and 75 µg/mL without activation (24-hour exposure)

Metabolic activation: With [ ]; Without [ ]; With and Without [X];
No data [ ]

Results: Negative without metabolic activation with a 24-hour exposure and with metabolic activation with a 4-hour exposure; equivocal without metabolic activation with a 4-hour exposure.

Cytotoxicity conc.: With metabolic activation (4-hr): None
Without metabolic activation (4- & 24-hr): ≥ 500 µg/mL

Genotoxic effects:		+	?	-
	With metabolic activation (4-hr):	[ ]	[ ]	[X]
	Without metabolic activation (4-hr):	[ ]	[X]	[ ]
	Without metabolic activation (24-hr):	[ ]	[ ]	[X]

Method: OECD Test Guideline 476 (1998)

GLP: Yes [X] No [ ] ? [ ]

Test Substance: Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); from General Electric Plastics; Purity: > 99.9%.

Remarks: Description of test procedure: The preliminary toxicity assay was used to establish the optimal dose levels for the mutagenesis assay. L5178Y cells were exposed to the solvent alone and nine concentrations of test article ranging from 0.15 to 1500 μg/mL in both the absence and presence of S9-activation with a 4-hour exposure and without activation with a 24-hour exposure. Cell population density was determined 24 and 48 hours after the initial exposure to the test article. The cultures were adjusted to $3x10^5$ cells/mL after 24 hours only. Toxicity was measured as suspension growth of the treated cultures relative to the growth of the solvent control cultures after 48 hours.

The mutagenesis assay was carried out by combining $6x10^6$ L5178Y/$TK^{+/-}$ cells, medium or S9 activation mixture and 100 μL dosing solution of test or control article in solvent or solvent alone and incubated for 4 (with and without activation) or 24 (without activation) hours. The positive controls were treated with MMS (at 10 and 20 μg/mL for the 4-hour exposure or 2.5 and 5.0 μg/mL for the 24-hour exposure) and 7,12-DMBA (2.5 and 4 μg/mL).

Expression of the mutant phenotype: For expression of the mutant phenotype, the cultures were counted and adjusted to $3x10^5$ cells/mL at approximately 24 and 48 hours after treatment. Cultures with less than $3x10^5$ cells/mL were not adjusted. For expression of the $TK^{-/-}$ cells, two flasks per culture were cloned for TFT (trifluorothymidine, the selective agent) or VC (viable count). The cells were diluted in cloning medium to concentrations of $3x10^6$ cells/100/mL for the TFT flask and 600 cells/100mL for the VC flask. Cells were plated and incubated for 10-14 days.

Scoring procedures: The VC plates were counted for the total number of colonies per plate and the total relative growth determined. The TFT-resistant colonies were counted for each culture with ≥ 20% total relative growth (including at least one concentration with ≥ 10% but ≤ 20% total growth). The diameters of the TFT-resistant colonies for the positive and solvent controls and, in the case of a positive response, the test article-treated cultures were determined over a range of approximately 0.2 to 1.1 mm.

Evaluation of results: The cytotoxic effects of each treatment condition were expressed relative to the solvent-treated control for suspension

growth over two days post-treatment and for total growth (suspension growth corrected for plating efficiency at the time of selection). The mutant frequency (number of mutants per 10^6 surviving cells) was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies in the three corresponding VC plates and multiplying by the dilution factor ($2x10^{-4}$) then multiplying by 10^6. In evaluation of the data, increases in mutant frequencies that occurred only at highly toxic concentrations (i.e., less than 10% total growth) were not considered biologically relevant. The following criteria are presented as a guide to interpretation of the data: (1) A result was considered positive if a concentration-related increase in mutant frequency was observed and one or more dose levels with 10% or greater total growth exhibited mutant frequencies of ≥100 mutants per 10^6 clonable cells over the background level; (2) A result was considered equivocal if the mutant frequency in treated cultures was between 55 and 99 mutants per 10^6 clonable cells over the background level; and (3) A result was considered negative if the mutant frequency in treated cultures was fewer than 55 mutants per 10^6 clonable cells over the background level.

Criteria for a Valid Test: For the negative control, the spontaneous mutant frequency of the cultures must be within 20 to 120 TFT-resistant mutants per 10^6 surviving cells. The cloning efficiency of the solvent control group must be greater than 50%. For positive controls, at least one concentration of each positive control must exhibit mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level. The colony size distribution for the MMS positive control must show an increase in both small and large colonies. For the BPA-BI cultures, a minimum of four analyzable concentrations with mutant frequency data was required.

Activation system: Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice.

Results: The maximum dose tested in the preliminary toxicity assay was 1500 μg/mL. Visible precipitate was present at ≥ 150 μg/mL in treatment medium. No visible precipitate was present at concentrations of ≤ 50 μg/mL in treatment medium. The osmolality of the solvent control was 462 mmol/kg and the osmolality of the highest soluble dose, 50 μg/mL, was 439 mmol/kg. Suspension growth relative to the solvent controls was 21% without activation and a 4-hour exposure, 73% with S9 activation and a 4-hour exposure, and 50% without activation and a 24-hour exposure at 1500 μg/mL. Based on the results of this preliminary test the concentrations selected for the mutagenesis assay were 0, 10, 25, 50, 75 and 100 μg/mL with and without activation for the 4-hour

exposure and 0, 5, 10, 25, 50 and 75 μg/mL for the 24-hour exposure without activation.

Results for cultures treated for four hours (initial assay): Visible precipitate was present at a concentration of 100 μg/mL. No visible precipitate was observed at concentrations ≤ 75 μg/mL. In the non-activated system, suspension growth ranged from 73 to 101%. In the activated system, suspension growth ranged from 92 to 111%. One non-activated cloned culture (4-hour exposure) exhibited a mutant frequency of ≥ 100 mutants per 10^6 clonable cells greater than the solvent control. Two non-activated and one activated cloned cultures (4-hour exposure) exhibited mutant frequencies between 55 and 99 mutants per 10^6 clonable cells. A dose-response was not observed in either non-activated or activated systems. The total growths ranged from 37 to 108% for the non-activated cultures at concentrations of 10 to 100 μg/mL and 40 to 87% for the S9-activated cultures at concentrations of 10 to 100 μg/mL. The results of the initial 4-hour exposure assay in the non-activated system were considered equivocal and negative in the activated system. Because no unique metabolic requirements were known about the test article, only an extended treatment assay was performed in the absence of S9 for a 24-hour exposure period.

Results for cultures treated for 24 hours (extended treatment assay): Visible precipitate was present at 75 μg/mL. No visible precipitate was observed at concentrations ≤ 50 μg/mL Cultures treated with concentrations of 0, 5, 10, 25, 50 and 75 μg/mL were cloned and produced a range in suspension growth of 57 to 98%.
No cloned culture exhibited a mutant frequency of between 55 and 99 mutants per 10^6 clonable cells over that of the solvent control. A dose-response trend was not observed. The total growths ranged from 53 to 156% at concentrations of 5 to 75 μg/mL.
The TFT-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2 to 1.1 mm. The colony sizing for the MMS positive control yielded the expected increase in small colonies, verifying the adequacy of the methods used to detect small colony mutants.

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells Treated with BPA-BI in the Absence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	60	60	30	50 ± 14	157	80	210	149 ± 53	67	--	--
0 (solvent)	2	39	18	+	29 ± 9	171	225	122	173 ± 42	33	--	--
Mean Solvent Mutant Frequency = 50												
10	A	54	65	32	50 ± 14	181	164	170	172 ± 7	59	9	108
10	B	21	28	30	26 ± 4	210	165	166	180 ± 21	29	-21	107
25	A	57	47	66	57 ± 8	160	107	155	141 ± 24	81	31	82
25	B	24	27	26	26 ± 1	170	156	109	145 ± 26	35	-15	82
50	A	92	55	71	73 ± 15	153	194	175	174 ± 17	84	33	98
50	B	53	65	51	56 ± 6	65	156	94	105 ± 38	107	57	59
75	A	66	53	63	61 ± 6	179	109	179	156 ± 33	78	28	84
75	B	90	93	67	83 ± 12	67	132	146	115 ± 34	145	95	61
100*	A	77	78	89	81 ± 5	190	157	175	174 ± 13	93	43	91
100*	B	76	73	51	67 ± 11	48	135	64	82 ± 38	162	112	37
Positive Control - Methyl Methanesulfonate (µg/mL)												
10	--	57	51	74	61 ± 10	27	114	116	86 ± 41	142	92	37
20	--	106	55	65	75 ± 22	18	47	45	37 ± 13	411	361	9

Solvent = DMSO
* Precipitating concentration
+ = Culture lost
[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200
[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls
[c] % Total growth = (% suspension growth x % cloning growth) / 100

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells Treated with BPA-BI in the Presence of Exogenous Metabolic Activation
Initial Assay (4-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	48	32	13	31 ± 14	122	175	178	158 ± 26	39	--	--
0 (solvent)	2	21	13	+	17 ± 3	170	140	90	133 ± 33	25	--	--
Mean Solvent Mutant Frequency = 32												
10	A	35	24	47	35 ± 9	113	87	110	103 ± 12	68	36	76
10	B	18	31	21	23 ± 6	74	76	113	88 ± 18	53	21	66
25	A	52	32	18	34 ± 14	164	117	77	119 ± 36	57	25	86
25	B	40	18	30	29 ± 9	160	84	112	119 ± 31	49	17	87
50	A	42	47	25	38 ± 9	170	100	67	112 ± 43	68	35	81
50	B	18	15	52	28 ± 17	66	77	116	86 ± 21	66	33	60
75	A	+	58	38	48 ± 8	92	61	117	90 ± 23	107	74	61
75	B	18	26	22	22 ± 3	42	53	70	55 ± 12	80	48	40
100*	A	45	17	31	31 ± 11	143	81	86	103 ± 28	60	28	65
100*	B	24	21	+	23 ± 1	100	84	173	119 ± 39	38	5	79
Positive Control - 7,12 Dimethylbenz(a)anthracene (µg/mL)												
2.5	--	78	86	+	82 ± 3	57	64	96	72 ± 17	227	194	32
4	--	177	195	130	167 ± 27	77	100	120	99 ± 18	338	306	26

Solvent = DMSO
* Precipitating concentration
+ = Culture lost
[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200
[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls
[c] % Total growth = (% suspension growth x % cloning growth) / 100

Cloning Data for L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells Treated with BPA-BI in the Absence of Exogenous Metabolic Activation Extended Treatment Assay (24-hour exposure)

Dose Level (µg/mL)	Replicate	TFT Colonies				VC Colonies				Mutant Freq.[a]	Induced Mutant Freq.[b]	% Total Growth[c]
		Counts			Mean	Counts			Mean			
0 (solvent)	1	101	52	53	69 ± 23	210	166	137	171 ± 30	80	--	--
0 (solvent)	2	44	54	76	58 ± 13	203	100	142	148 ± 42	78	--	--
Mean Solvent Mutant Frequency = 79												
5	A	42	33	90	55 ± 25	181	139	116	145 ± 27	76	-4	82
5	B	58	96	91	82 ± 17	111	135	143	130 ± 14	126	47	78
10	A	47	74	78	66 ± 14	137	166	143	149 ± 12	89	10	53
10	B	45	32	+	39 ± 5	112	105	204	140 ± 45	55	-24	86
25	A	80	61	120	87 ± 25	240	227	270	246 ± 18	71	-8	126
25	B	46	98	81	75 ± 22	244	271	234	250 ± 16	60	-19	144
50	A	84	46	74	68 ± 16	249	159	196	201 ± 37	68	-12	115
50	B	51	40	126	72 ± 38	130	137	256	174 ± 58	83	4	93
75*	A	91	76	54	74 ± 15	281	263	219	254 ± 26	58	-21	156
75*	B	40	38	77	52 ± 18	222	212	236	223 ± 10	46	-33	135
Positive Control - Methyl Methanesulfonate (µg/mL)												
2.5	--	76	266	123	155 ± 81	113	107	124	115 ± 7	270	191	67
5	--	242	150	275	222 ± 53	92	124	90	102 ± 16	436	357	44

Solvent = DMSO

* Precipitating concentration

+ = Culture lost

[a] Mutant frequency (per 10^6 surviving cells) = (Average # TFT colonies / average # VC colonies) x 200

[b] Induced mutant frequency (per 10^6 surviving cells) = mutant frequency - average mutant frequency of solvent controls

[c] % Total growth = (% suspension growth x % cloning growth) / 100

Conclusions: All criteria for a valid study were met as described in the protocol. The results of the L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenesis Assay indicate that, under the conditions of this study, the mutagenicity of BPA-BI was concluded to be negative without activation with a 24-hour exposure and with activation with a 4-hour exposure. The 4-hour exposure without activation was equivocal based on the criteria established in the protocol (Author of the Report). However, this response occurred at precipitating concentrations and was not repeated in the more stringent 24-hour test. Therefore, BPA-BI is considered to be negative in this assay (Sponsor of the Study).

Reference: San, R.H.C. and Clarke, J.J. (2003). Unpublished Report No. AA79BU.704.BTL entitled "*In vitro* mammalian cell gene mutation test (L5178Y/TK$^{+/-}$ mouse lymphoma assay)" dated November 22, 2004 for

	General Electric Company, Pittsfield, MA, USA; from BioReliance, Rockville, MD, USA.
Reliability:	(Klimisch Code 1) Valid without restrictions.

16.0 REPEATED DOSE TOXICITY

Species/Strain:	Rat/Sprague-Dawley Crl:COBS®, CD®, (SD) Br
Sex:	Female [ ]; Male [ ]; Male/Female [X]; No data [ ]
Route of Administration:	Oral, Dietary feed
Exposure Period:	30 days
Frequency of Treatment:	Daily
Post Exposure Observation Period:	None
Dose:	0, 1, 2, and 4% of BPA-BI in basal diet (approximately 0, 646 – 765, 1277 – 1490, and 2750 – 3160 mg/kg/day, respectively)
Control Group:	Yes [X] No [ ] No data [ ] Concurrent no treatment [X] Concurrent vehicle [ ] Historical [ ]
NOAEL:	4% (approximately 2750 to 3160 mg/kg/day)
LOAEL:	> 4%
Method:	Not stated.
Year:	1982
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); Lot UI-82-3 from General Electric Company; Purity: See "Chemical Identity and Use Information" section.
Remark:	Test procedure: Groups of 10 rats/sex were fed BPA-BI at concentrations of 0, 1, 2 and 4%. All rats were observed for mortality twice each day. Clinical signs and body weights were recorded at initiation and weekly thereafter. Food consumption was recorded weekly. After 31 days of treatment, all surviving rats were weighed, killed and a gross necropsy was performed. At necropsy, the liver and kidneys of each animal were weighed and organ to body weight ratios determined. The following tissues were preserved from all animals: brain, pituitary, thoracic spinal cord, eyes, salivary glands, thyroid, parathyroids, thymus, trachea, esophagus, lung, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, colon, cecum, mesenteric lymph node, urinary bladder, testes with epididymides and prostate (males), ovaries and uterus (females), femur, costal bone marrow, skeletal muscle, and all gross lesions. Microscopic evaluation was conducted on sections of the lungs, liver, brain and kidneys from rats of all treatment groups. Reproductive organs were not evaluated histologically. The following statistical tests were utilized to evaluate body weight changes, total food consumption and organ weights: Bartlett's test for homogeneity of variance and one-way classification analysis of variance (ANOVA). Since the ANOVA proved to be not significant for all of the analyses, no other tests were performed. All analyses were performed at the 5% one-tailed probability level.

Results:	No deaths occurred during the study. No compound-related clinical observations were noted throughout the study. Body weight and food consumption data of the compound-treated males and females were generally comparable to those of their respective controls. Individual and mean terminal body weights, absolute organ weights and organ weights relative to terminal body weight were not affected by treatment. No compound-related organ or tissue changes were evident macroscopically or microscopically.
Reference:	Burdock, G.A. and Kundzins, W. (1982). Unpublished Report No. 349-262 entitled "Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA", dated December 17, 1982 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and Burdock, G.A. (1984). Unpublished addendum to Final Report No. 349-262 entitled "Thirty-Day Subchronic Oral Toxicity Study in Rats, BPA-BI and BPA-DA", dated December 22, 1984 for General Electric Company, Mount Vernon Indiana, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.
Reliability:	(Klimisch Code 2) Valid with restrictions. Minimal data collected for a repeat dose study.

17.0 REPRODUCTIVE TOXICITY

Type:	Fertility [ ] One generation study [ ] Two generation study [ ] Other [X]
Species/Strain:	Rat/CD® (Sprague-Dawley)
Sex:	Female [ ] Male [ ] Male/Female [X] No data [ ]
Route of Administration:	Oral, gavage
Exposure Period:	Males = 4 weeks (2 week pre-breeding, 2 weeks mating) Females = ~7 weeks (2 weeks prebreed, 2 weeks mating, 3 weeks gestation, and lactation through pnd 4)
Frequency of Treatment:	Daily
Premating Exposure Period:	2 weeks
Duration of Test:	F0 generation males 4 weeks F0 generation females postnatal day 4
Dose:	0, 100, and 100 mg/kg/day
Control Group:	Yes [X]; No [ ]; No data [ ]; Concurrent no treatment [ ] Concurrent vehicle [X] Historical [ ]
NOAEL Parental:	Male: 1000 mg/kg/day Female: 100 mg/kg/day
NOAEL Reproduction:	>1000 mg/kg/day (males and females)
NOAEL F1 Offspring:	>1000 mg/kg/day (males and females)
Method:	OECD Test Guideline 421 (1998); due to the limited toxicity observed in previous studies for BPA-BI, only two BPA-BI dose groups were used.
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); Lot U10058 from General Electric Company; Purity: >99.9%.

Remark:

Test procedure: Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered BPA-BI orally by gavage at 0, 100, and 1000 mg/kg/day at a dose volume of 5 mL/kg/day in corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure and two weeks of mating for F0 male and female parental animals. F0 females continued to be dosed for three weeks of gestation and through post natal day (pnd) 3. Body weights for the F0 males and females were recorded weekly during the prebreed and mating periods for both sexes and for F0 females during gestation. During lactation, F0 female body weights were obtained on pnd 0 and 4. Feed consumption was recorded weekly for the F0 males and females during the prebreed period, but not during the mating period. Feed consumption was recorded for the F0 females during gestation and through pnd 4 of lactation. Clinical signs were recorded at least once daily for all animals. After the two-week prebreed exposure period, animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation. F0 males were sacrificed following the breeding period (after 28 days of dosing). F0 females with litters were sacrificed on pnd 4 and F0 females that did not produce a litter were sacrificed on gestation day (gd) 26 or 26 days after mating.

On the day of birth (pnd 0), all live F1 pups were counted, sexed, weighed and examined as soon as possible. All stillborn pups or pups that died on the date of birth were sexed and counted. All pups were examined daily from birth through pnd 4 for survival and physical abnormalities. Any pups dying during lactation were necropsied, if possible. On pnd 4, all live pups were examined sexed and weighted, then euthanized and discarded without further evaluation.

At the F0 parental animal necropsy, the following tissues were weighed and retained: testes, epididymides, prostate, seminal vesicles, ovaries, uterus. All gross lesions were also retained. Histopathology was performed on all retained reproductive tissues for the high dose and control males and females with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure (10/sex/group). The uteri from the F0 females that failed to produce a litter by gd 26 or by 26 days post-mating were stained with potassium ferricyanide for confirmation of pregnancy.

Results:

The following is a discussion of the F0 adult systemic toxicity, F0 parental reproductive toxicity, and F1 offspring toxicity. Summary data tables are also provided. The discussion focuses on treatment-related effects. Other changes noted in the tables were considered random, due to biological variation, and not related to treatment.

F0 Adult Systemic Toxicity: A table follows the results write-up that provides a summary of F0 adult systemic toxicity.

Males: There were no treatment-related deaths for the F0 males. For parental males, minor systemic toxicity was present at 1000

mg/kg/day, expressed as decreased body weight change from sd 14-21.There was no change in feed consumption across dose groups and study days. Clinical observations were restricted to post-dose rooting during the dosing phase of the study. "Post-dose rooting" was defined as the animal digging or moving its bedding with its snout postdosing, possibly to mitigate the adverse taste. Post-dose rooting and salivation are considered to be behavioral responses to taste aversion to the dosing formulations and not a toxic sign. Since there was a dose-related increase in the incidence of post-dose rooting (0, 0 and 3 males in the 0, 100 and 1000 mg/kg/day groups, respectively), it is presumed that the increasing concentrations of BPA-BI across groups caused the adverse taste reaction. At scheduled sacrifice, mean body weights of F0 males were equivalent across the three groups. Absolute and relative weights for paired testes, paired epididymides, prostate and paired seminal vesicles with coagulating glands were also equivalent across the groups. There were no treatment-related macroscopic or microscopic findings. There were a limited number of histopathologic changes observed in both control and high dose males, which are typical of the spontaneous type of microscopic pathology observed in this age and strain of rat.

Females: There were no treatment-related deaths for the F0 females. There were no significant changes in F0 female body weights or feed consumption during the prebreed or mating period; however, there was a decrease in body weight change from sd 0-14 in the 1000 mg/kg/day group. There were no treatment related clinical observations for the F0 females during the course of the study (i.e. prebreed, mating period, gestation and lactation). One female at 100 mg/kg/day bit her tongue prior to being dosed during the prebreed period and continued to have audible respiration throughout the study. Reductions in F0 maternal body weights occurred on gd 0 for 100 and 1000 mg/kg/day, gd 7 for 100 mg/kg/day, and gd 14 and gd 20 for 100 and 1000 mg/kg/day. F0 female body weight change was decreased from gd 14 to 20 at 100 mg/kg/day and was not affected at any other dose or period during gestation. The differences in body weight were not dose related and did not exceed 10% reduction from the control group value. Further, the difference for the 100 mg/kg/day group occurred immediately on gd 0, even though there was no difference in body weight for the group on sd 14. Examination of the data shows that the females that were not pregnant were heavier than the pregnant animals in the 100 mg/kg/day group. In addition, the body weights for sd 21 indicated that the nonpregnant animals at 100 mg/kg/day were heavier the nonpregnant controls. Thus, the body weight difference for the 100 mg/kg/day group during gestation was the result of the subgrouping (pregnant and nonpregnant) and was not a result of BPA-BI toxicity. Also, at pnd 0, the body weights for the F0 dams were equivalent across all groups. There were no significant changes in F0 maternal body weights or body weight change at any dose throughout lactation. Feed consumption was unaltered during gestation and lactation. At scheduled sacrifice, mean body weights of F0 females were

equivalent across the three groups. The absolute weight and the weight relative to final body weight of the uterus (with cervix and vagina) and paired ovary were equivalent across the dose groups. There were no treatment related microscopic or macroscopic findings.

Summary of F0 Adult Systemic Toxicity – Key Parameters and Statistically Significant Differences

		F0		
Bisphenol A Bisimide (mg/kg/day)		**0**	**100**	**1000**
F0 MALES				
Deaths		0/10	0/10	0/10
Body Weights		---	---	---
Weight Change:				
sd 7 to 14		---	---	↓
Feed Consumption:	g/day	---	---	---
	g/kg/day	---	---	---
Necropsy:				
Final Body Weight at Necropsy		---	---	---
Organ weights		---	---	---
F0 FEMALES				
Deaths		0/10	0/10	0/10
Prebreed, Mating, and Postmating (sd 0-42) Exposure				
Body Weights		---	---	---
Weight Change				
sd 0 to 14		---	---	↓
Feed Consumption:	g/day	---	---	---
	g/kg/day	---	---	---
Gestation				
Body Weights				
gd 0		---	↓	↓
gd 7		---	↓	---
gd 14		---	↓	↓
gd 20		---	↓	↓
Weight Change				
gd 7 to 14		---	↓	---
Feed Consumption:	g/day	---	---	---
	g/kg/day	---	---	---
Lactation (pnd 0-4)				
Body Weights		---	---	---
Weight Change		---	---	---
Feed Consumption:	g/day	---	---	---
	g/kg/day	---	---	---
Necropsy				
Final Body Weight at Necropsy		---	---	---
Organ weights		---	---	---

↓ = statistically significant decrease at $p<0.05$

--- = no statistically significant difference

Results (Continued): F0 Parental Reproductive Toxicity: The following Table presents a summary of F0 parental reproductive toxicity. During the post mating period, there were 8, 7, and 9 females in the 0, 100, and 1000 mg/kg/day dose groups, respectively, that were sperm positive; however, the total number of females confirmed pregnant at study completion was 10, 8 and 10, respectively. One female in the 100 mg/kg/day group had an implantation site but did not deliver. There were no significant effects of exposure to BPA-BI on F0 fertility, mating or pregnancy indices during the production of F1 offspring; these indices were equivalent across the groups. There were no significant differences across groups for percent implantations or postimplantation loss per litter or the number of dead pups at birth.

Summary of F0 Parental Male and Female Reproductive Toxicity

	F0		
Bisphenol A Bisimide (mg/kg/day)	**0**	**100**	**1000**
FEMALES			
No. Females on Study	10	10	10
No. Females Paired	10	10	10
No. Females that Mated	10	8	10
Mating Index (# females mated/# females paired)	100.0	80.0	100.0
No. Pregnant Females	10	8	10
Fertility Index (# pregnant females/# females that mated)	100.0	100.0	100.0
No. of Females with Live Litters (pnd 0)	10	7[a]	10
Gestational Index (# females with live litters/# females pregnant)	100.0	87.5	100.0
MALES			
No. Males on Study	10	10	10
No. Males Paired	10	10	10
No. Males that Mated	10	8	10
Mating Index (# males mated/# males paired)	100.0	80.0	100.0
No. Males Siring Litters	10	8	10
Fertility Index (# males siring litters/# males that mated)	100.0	100.0	100.0
Pregnancy Index (# females with live litters/# males that mated)	100.0	87.5	100.0
Precoital Interval (days)	2.9 ± 0.6	2.3 ± 0.6	1.8 ± 0.4
Gestational Length (days)	21.9 ± 0.3	22.0 ± 0.0	22.1 ± 0.1
No. Live Litters			
Postnatal Day 0	10	7	10
Postnatal Day 4	10	7	10
No. Corpora Lutea per Dam	13.20 ± 0.85	13.00 ± 2.03	13.40 ± 0.58
% Preimplantation Loss per Litter	11.87 ± 6.88	7.47 ± 5.10	6.05 ± 4.15
Average No. Implantation Sites per Litter	13.20 ± 1.13	12.25 ± 1.77	14.40 ± 1.06
% Postimplantation Loss per Litter	2.91 ± 2.10	18.60 ± 11.92	4.66 ± 1.50

	F0		
Bisphenol A Bisimide (mg/kg/day)	**0**	**100**	**1000**
Average No. of Live Pups on Postnatal Day 0	13.2 ± 1.3	13.4 ± 1.3	13.8 ± 1.1
Average No. of Dead Pups on Postnatal Day 0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Average Total Number of Pups on Postnatal Day 0	13.4 ± 1.3	13.4 ± 1.3	13.8 ± 1.1
Stillbirth Index (# dead on pnd 0/total # on pnd 0)	1.1 ± 1.1	0.0 ± 0.0	0.0 ± 0.0
Live birth Index (# live on pnd 0/total # on pnd 0)	98.9 ± 1.1	100.0 ± 0.0	100.0 ± 0.0
4 Day Survival Index (# surviving 4 days/ # live on pnd 0)	100.0 ± 0.0	99.0 ± 1.0	98.1 ± 1.4

[a] One female had 1 implantation site at necropsy but did not deliver a litter.

Results (Continued): F1 Offspring Toxicity: The following Table presents a summary of F1 offspring toxicity parameters. There were 10, 7 and 10 live litters on pnd 0 and 4 at 0, 100 and 1000 mg/kg/day, respectively. The following parameters were unaffected by treatment: live birth, stillborn and survival (pnd 0-4) indices, mean number of live pups per litter for pnd 0 and 4, mean pup body weights, sex ratio and pup mortality. Non-treatment-related necropsy findings were reported for F1 pups found dead or euthanized moribund on pnd 0-4 included pups that died on pnd 0 (2 at 0 mg/kg/day) and pnd 4 (2 at 1000 mg/kg/day). Three pups were too autolyzed for evaluation and one had no findings.

Summary of F1 Offspring Toxicity

	F1		
Bisphenol A Bisimide (mg/kg/day)	**0**	**10**	**100**
No. Live Litters			
Postnatal Day 0	10	7	10
Postnatal Day 4	10	7	10
Average No. of Live Pups per Litter (pnd 0)	13.2 ± 1.3	13.4 ± 1.3	13.8 ± 1.1
Average No. of Live Pups per Litter (pnd 4)	13.2 ± 1.3	13.4 ± 1.3	13.5 ± 1.0
Average Pup Body Weight (g) per Litter (pnd 0)	6.71 ± 0.24	6.44 ± 0.32	6.66 ± 0.13
Average Male Body Weight (g) per Litter (pnd 0)	6.85 ± 0.26	6.54 ± 0.29	6.89 ± 0.15
Average Female Body Weight (g) per Litter (pnd 0)	6.59 ± 0.24	6.34 ± 0.33	6.47 ± 0.14
Average Pup Body Weight (g) per Litter (pnd 4)	10.93 ± 0.66	10.55 ± 0.78	10.45 ± 0.33
Average Male Body Weight (g) per Litter (pnd 4)	11.20 ± 0.76	10.75 ± 0.77	10.78 ± 0.40
Average Female Body Weight (g) per Litter (pnd 4)	10.74 ± 0.62	10.32 ± 0.74	10.17 ± 0.30
% Male Pups per Litter (pnd 0)	47.5 ± 5.3	52.6 ± 3.7	45.8 ± 5.5
% Male Pups per Litter (pnd4)	48.4 ± 5.3	52.1 ± 3.7	46.7 ± 5.4

Conclusion: BPA-BI administered by gavage once daily at 0, 100 and 1000 mg/kg/day to parental F0 CD® (SD) rats, 10/sex/group, through prebreed, mating , gestation and lactation, did not results in adverse effects in males at either dose. In the F0 females, body weight change was decreased during the prebreed period that was reflected in decreased body weight during gestation at 1000 mg/kg/day. There was no evidence of reproductive

	toxicity in the F0 females at either dose nor any toxicity in the F1 offspring observed postnatally. At necropsy, no treatment-related macroscopic or microscopic findings were present. Based on these results, the NOAEL for the F0 male and female systemic toxicity was 1000 and 100 mg/kg/day, respectively. The NOAEL for F0 reproductive toxicity was > 1000 mg/kg/day for both sexes. The NOAEL for F1 toxicity was >1000 mg/kg/day for both sexes.
Reference:	Tyl, R.W., Myers, C.B. and Marr, M.C. (2005). Unpublished Report No. 08627.004.200 entitled "Reproductive/Developmental Toxicity Screening Test of Bisphenol A Bisimide (BPA-BI; CAS No. 54395-52-7) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (OECD 421)", dated October 12, 2005 for General Electric Company, Pittsfield, MA from RTI International, Research Triangle Park, NC, USA.
Reliability:	(Klimisch Code 1) Valid without restrictions.

18.0 DEVELOPMENTAL TOXICITY/TERATOGENICITY

18.1	
Species/Strain:	Rabbit, New Zealand White
Sex:	Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration:	Oral (gavage)
Duration of Test:	29 days
Exposure Period:	Days 6 through 18 of gestation
Frequency of Treatment:	Daily
Dose:	1000 mg/kg/day
Control group:	Yes [X] No [ ] No data [ ] Concurrent no treatment [ ] Concurrent vehicle [X] Historical [ ] Positive Control (thalidomide)
NOEL Maternal Toxicity:	> 1000 mg/kg/day
NOEL Teratogenicity:	> 1000 mg/kg/day
Year:	1983
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); Lot UI-82-3 from General Electric Company; Purity: See "Chemical Identity and Use Information" section.
Method:	Not stated.
Remark:	Test procedure: Ninety mature New Zealand White female rabbits were obtained from Dutchland Laboratory Animals, Inc., Denver, PA for use in this study. The animals were acclimated for a minimum of 22 days prior to the initiation of the study. During the period of acclimation, the rabbits were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rabbit ration (Purina lab Rabbit Chow®) and tap water *ad libitum*. The environment of the study room was maintained at 70-78 ºC, relative humidity of 53-86% and a 12-hour light/dark cycle. At Day 0 of gestation, the body weights ranged from 2845 to 4700 grams. The animals were artificially

inseminated with sperm from the laboratory breeding stock five hours after induction of ovulation with chorionic gonadotropin. Five groups were included in this study; for the purposes of this summary, only three groups (control, positive control, and BPA-BI treated) will be discussed. Sixteen animals per group (to obtain at least 12 pregnant) were treated with vehicle (0.5% carboxymethyl cellulose), positive control (thalidomide; 150 mg/kg/day) or BPA-BI (1000 mg/kg/day). Thalidomide and BPA-BI were suspended in vehicle to provide dose volumes of 1.5 and 4.0 mL/kg, respectively. Control dose volume was 4.0 mL/kg. The dose was administered from gestation day (gd) 6 through 18, approximately the same time each day, and was based on each individual body weight on gd 6 (starting on gd 11, two animals in the control group, four animals in the thalidomide group and five animals in the BPA-BI group were dosed based on gd 11 body weight). All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights were recorded on gd 0, 6, 11, 15, 19, and 29. Individual food consumption was recorded weekly. On gd 29, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, weighed and measured from the frontal-parietal suture to the base of the tail (crown-rump distance). Caesarean sections were also performed on dams that were found dead, sacrificed moribund or sacrificed due to early delivery. The number of corpora lutea, implantations, resorptions and live or dead fetuses was recorded.
Visceral Examination of Fetuses: The unfixed fetuses underwent visceral examination according to the method of Staples. All of the fetuses were opened by longitudinal incision, the sex determined and examined grossly both externally and internally. Major organs were inspected *in situ* with special attention to the heart and major blood vessels. The heads of approximately one-third of the fetuses were removed, fixed in Bouin's solution, sectioned by Wilson's freehand sectioning technique for examination of the eyes, palate, nasal septum and brain. The prepared sections were then re-examined against a light box with the aid of magnification.
Skeletal Examination of Fetuses: Following visceral examination, all fetuses (minus the head for approximately one-third of the fetuses) were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies. Examinations were performed with the aid of magnification on a light box.

Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Games and Howell modification of the Tukey-Kramer honestly significant difference test was used to compare groups. Pregnancy rates were analyzed by Fisher's exact test. External, visceral, and skeletal anomalies were evaluated by a multiple proportions test. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights and lengths with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.

Range-finding study: A range-finding study was conducted to select the dose used in this study. Four non-pregnant New Zealand White rabbits were dosed with BPA-BI at 2000 mg/kg/day for the first six days and, following a three-day rest period, the dose was changed to 1000 mg/kg/day for the remaining seven days of the study. One of the four rabbits died on Day 4. Compound-related clinical signs included depression, slight depression and anorexia. Two of the surviving animals lost weight throughout the study. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results: Weight loss was observed in the thalidomide-treated group during the treatment period. Statistical evaluation of body weight change did not, however, reveal any significant differences between treated and control groups. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
Number of dams	16	16	16
Number pregnant	14	16	16
Pregnancy rate (%)	88	100	100
Number dams surviving to gd 29 (survival rate)	13 (93%)	15* (100%)	15 (94%)

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
Mean number of			
Corpora lutea	13.4	12.2	10.5
Implantations	9.4	8.3	7.8
Resorptions-total	1.2	5.3	1.1
Fetuses – live	7.5	3.4	6.4
– dead	0.5	0	0
Indices (mean per litter)			
Implantation efficiency (%)	73.6	68.1	73.1
Incidence of resorption (%)	17.2	61.0	17.4
Incidence of fetal mortality (%)	3.8	0	0
Incidence of fetal viability (%)	79.2	39.1	82.6
Live fetuses			
Mean body weight (g) – males	40.91	38.36	44.04
– females	39.90	37.60	44.57
Mean length (cm) – males	9.49	9.03	9.48
– females	9.33	8.92	9.63
Percent Males	51.5	58.0	47.8
Mean uterine weights – gravid (g)	485.3	228.3	406.5

* One animal died accidentally on gd 8

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
External			
# of litters examined	12	11	14
# of litters with anomalous fetuses	2	10*	0
% of litters with anomalous fetuses	16.7	90.9	0
Mean values (per litter)			
# of fetuses with variants	0	0.5	0
Incidence of variants (%)	0	14.4	0
# of fetuses with anomalies	0.3	2.7	0
Incidence of anomalies (%)	2.4	64.1	0
Visceral – Fetal Heads			
# of litters examined	12	9	13
# of litters with anomalous fetuses	0	3	0
% of litters with anomalous fetuses	0	33.3	0
Mean values (per litter)			
# of fetuses with variants	0	0.1	0
Incidence of variants (%)	0	3.7	0
# of fetuses with anomalies	0	0.4	0
Incidence of anomalies (%)	0	16.7	0
Visceral – Torso and Limbs			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	8*	1
% of litters with anomalous fetuses	0	72.7	7.1

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	Thalidomide (Positive Control)	BPA-BI (1000 mg/kg/day)
Mean values (per litter)			
# of fetuses with variants	0.8	2.9	0.6
Incidence of variants (%)	11.1	63.9	9.3
# of fetuses with anomalies	0	1.5	0.1
Incidence of anomalies (%)	0	38.8	1.8
Skeletal – Skulls			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	2	0
% of litters with anomalous fetuses	0	18.2	0
Mean values (per litter)			
# of fetuses with variants	0.5	1.6	0.1
Incidence of variants (%)	11.3	60.9	3.4
# of fetuses with anomalies	0	0.2	0
Incidence of anomalies (%)	0	11.4	0
Skeletal – Torso and Limbs			
# of litters examined	12	11	14
# of litters with anomalous fetuses	0	10*	0
% of litters with anomalous fetuses	0	90.9	0
Mean values (per litter)			
# of fetuses with variants	0.6	3.9	0.4
Incidence of variants (%)	6.9	91.7	5.0
# of fetuses with anomalies	0	2.2	0
Incidence of anomalies (%)	0	54.4	0

* Statistically significantly different from vehicle control group ($p < 0.05$)

Conclusion: There were no differences from control in the thalidomide or BPA-BI dose groups for maternal, ovarian or uterine data. The thalidomide-treated group exhibited changes consistent with the known teratogenic effect of this compound. The thalidomide group may additionally have had an increase in resorptions and exhibited a possible fetotoxic effect as demonstrated by slightly decreased mean body weights and lengths of the fetuses. There were no effects on any fetal parameters from BPA-BI treatment. Based on the results of this study, BPA-BI is not a developmental toxin.

Reference: Burdock, G.A. (1983). Unpublished Report No. 349-267 entitled "Teratogenicity Study in Rabbits, PI, BPA-BI, BPA-DA", dated August 25, 1983 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and Burdock, G. A. (1982) Unpublished Report No. 349-263 entitled "Two-Week Pilot Toxicity Study in Rabbits: BPA-BI, BPA-DA, PI, and 4-NPI", dated August 20, 1982 for General Electric Company, Mount Vernon, IN, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability:	(Klimisch Code 1) Reliable without restrictions.
18.2	
Species/Strain:	Rat; Crl:CD®(SD)BR
Sex:	Female [X]; Male [ ]; Male/Female [ ]; No data [ ]
Route of Administration:	Oral (gavage)
Duration of Test:	20 days
Exposure Period:	Days 6 through 15 of gestation
Frequency of Treatment:	Daily
Dose:	1000 mg/kg/day
Control group:	Yes [X] No [ ] No data [ ] Concurrent no treatment [ ] Concurrent vehicle [X] Historical [ ]
NOEL Maternal Toxicity:	> 1000 mg/kg/day
NOEL Teratogenicity:	> 1000 mg/kg/day
Year:	1987
GLP:	Yes [X] No [ ] ? [ ]
Test Substance:	Bisphenol A Bisimide (BPA-BI; CAS RN 54395-52-7); from General Electric Company; Purity: See "Chemical Identity and Use Information" section.
Method:	Not stated.
Remark:	Test procedure: One hundred twenty, successfully mated Sprague-Dawley female rats, obtained from Charles River Breeding Laboratories, Inc., (Portage, MI), were used in this study. Prior to in-house breeding, the rats were examined for general health and appearance. The animals were uniquely identified by ear tag and provided commercial rat ration (Purina Certified Rodent Chow®) and tap water *ad libitum*. The environment of the study room was monitored daily and a 12-hour light/dark cycle was used. Animals were mated one male to one female for 17 days. The day that vaginal sperm or a copulation plug was observed was designated Day 0 of gestation. At Day 0 of gestation, the body weights ranged from 195 to 289 grams. Five groups were included in this study; for the purposes of this summary, only two groups (control, and BPA-BI-treated) will be discussed. Twenty-four animals per group were treated with vehicle (0.5% carboxymethyl cellulose) or BPA-BI (1000 mg/kg/day). Dose volume was 10.0 mL/kg. The dose was administered from gestation day (gd) 6 through 15, approximately the same time each day, and was based on the most recently recorded body weight. All of the animals were observed daily for mortality, moribundity and clinical signs. Body weights and food consumption were recorded on gd 0, 6, 8, 12, 16, and 20. On gd 20, the animals were sacrificed, examined for gross pathology of the external surface and viscera, and the uterus excised and weighed. The fetuses were taken by cesarean section and the following recorded for each litter: the number of corpora lutea per ovary; the number and placement of uterine implantation sites; live and dead fetuses; early and late resorptions; and any other abnormalities. Fetuses were removed from the placenta, individually identified, examined externally, and weighed.

Visceral Examination of Fetuses: Approximately one-third of the live fetuses were selected for visceral examination according to the method of Wilson.
Skeletal Examination of Fetuses: The remaining fetuses were eviscerated and placed in 95% ethyl alcohol. After fixation and dehydration, the skeletons were stained in a potassium hydroxide-alizarin red solution. The skull, vertebral column, rib cage, pectoral and pelvic girdles, long bones and extremities of each skeleton were examined for degree of ossification, bone alignment, and possible anomalies.
Statistical Analyses: Mean maternal body weight changes, food consumption, percentage data (implantations, resorptions and males), and fetal viability were analyzed in the following order: Levene's test for homogeneity of variance; if the variances proved to be homogeneous, the data were analyzed by one-way classification analysis of variance (ANOVA); if the variance proved to be heterogeneous, a series of transformations was performed until homogeneity was achieved followed by ANOVA. If ANOVA was significant, the Dunnett's test was used to compare groups. Pregnancy rates, clinical observations and fetal skeletal observations were analyzed by Cochran-Armitage and Fisher-Irwin Exact Tests. Analysis of covariance (ANCOVA) was used to analyze mean fetal weights with the litter used as the experimental unit. Levene's test and ANOVA were evaluated at the 5% one-tailed probability level. Control vs. treatment group mean comparisons were evaluated at the 5% two-tailed probability level.
Range-finding study: A range-finding study was conducted to select the dose used in this study. Five pregnant Sprague-Dawley rats were dosed with BPA-BI at doses of 150, 400 or 1000 mg/kg/day from days 6 through 15 of gestation. No maternal or fetal effects were observed at any dose. Based on this study, 1000 mg/kg/day was selected for the teratology study.

Results: No treatment-related effect on maternal body weight or clinical observations were observed in the BPA-BI treated group. No effects on food consumption or gross pathology of the dams were observed. The following tables summarize the fetal results:

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Number of dams	24	24
Number pregnant	23	24
Pregnancy rate (%)	96	100

Summary of Mean Ovarian, Uterine, and Litter Data

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Number dams surviving to gd 29 (survival rate)	24 (100%)	24 (100%)
Mean number of		
Corpora lutea	16.9	17.2
Implantations (% Efficiency)	14.8 (89)	14.9 (88)
Resorptions-total	0.9	0.7
Fetuses – live	14.0	14.3
– dead	0	0
Live fetuses		
Mean body weight (g) – males	3.6	3.6
– females	3.3	3.5
Mean uterine weights – gravid (g)	76.3	78.3

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
External Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	3	2
% of litters with anomalous fetuses	13	8.3
Fetal Incidence		
# of fetuses with variants	4	2
Incidence of variant (%)	1.2	0.6
External Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	1	0
% of litters with anomalous fetuses	4.3	0
Fetal Incidence		
# of fetuses with variants	1	0
Incidence of variant (%)	0.3	0
Soft Tissue Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	6	14
% of litters with anomalous fetuses	26	58
Fetal Incidence		
# of fetuses with variants	9	22
Incidence of variant (%)	9.2	21

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Soft Tissue Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	0	1
% of litters with anomalous fetuses	0	4.2
Fetal Incidence		
# of fetuses with variants	0	1
Incidence of variant (%)	0	1.0
Skeletal Variations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	23	24
% of litters with anomalous fetuses	100	100
Fetal Incidence	120	127
# of fetuses with variants	54	54
Incidence of variant (%)		

Summary of Mean Incidence of Abnormal Fetuses per Litter

Parameter	Control (Vehicle)	BPA-BI (1000 mg/kg/day)
Skeletal Malformations		
Litter Incidence		
# of litters examined	23	24
# of litters with anomalous fetuses	1	0
% of litters with anomalous fetuses	4.3	0
Fetal Incidence		
# of fetuses with variants	1	0
Incidence of variant (%)	0.4	0

Conclusion: There were no differences from control in the BPA-BI dose groups for maternal, ovarian or uterine data. There were no treatment-related effects on any fetal parameters from BPA-BI treatment. Based on the results of this study, BPA-BI is not a developmental toxin.

Reference: Morseth, S. L (1987). Unpublished Report No. HLA 349-265 entitled "Rat Teratology Study with BPA-DA, BPA-BI and NMP", dated March 5, 1987 for General Electric Company, Plastics Business Operations, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA; and

Burdock, G. A. (1985). Unpublished Report No. 349-326 entitled "Pilot Rat Teratology Study: NMP, BPA-BI, BPA-DA, and a Positive Control", dated October 29, 1985 for General Electric Company, Pittsfield, MA, USA; from Hazleton Laboratories America, Inc., Vienna, VA, USA.

Reliability: (Klimisch Code 1) Reliable without restrictions.